

# Proposals for the classification of human rhinovirus species C into genotypically assigned types



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Review

# Proposals for the classification of human rhinovirus species C into genotypically assigned types

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Human rhinoviruses (HRVs) are common respiratory pathogens associated with mild upper respiratory tract infections, but also increasingly recognized in the aetiology of severe lower respiratory tract disease. Wider use of molecular diagnostics has led to a recent reappraisal of HRV genetic diversity, including the discovery of HRV species C (HRV-C), which is refractory to traditional virus isolation procedures. Although it is heterogeneous genetically, there has to date been no attempt to classify HRV-C into types analogous to the multiple serotypes identified for HRV-A and -B and among human enteroviruses. Direct investigation of cross-neutralization properties of HRV-C is precluded by the lack of methods for *in vitro* culture, but sequences from the capsid genes (VP1 and partial VP4/VP2) show evidence for marked phylogenetic clustering, suggesting the possibility of a genetically based system comparable to that used for the assignment of new enterovirus types. We propose a threshold of 13 % divergence for VP1 nucleotide sequences for type assignment, a level that classifies the current dataset of 86 HRV-C VP1 sequences into a total of 33 types. We recognize, however, that most HRV-C sequence data have been collected in the VP4/VP2 region (currently 701 sequences between positions 615 and 1043). We propose a subsidiary classification of variants showing >10 % divergence in VP4/VP2, but lacking VP1 sequences, to 28 provisionally assigned types (subject to confirmation once VP1 sequences are determined). These proposals will assist in future epidemiological and clinical studies of HRV-C conducted by different groups worldwide, and provide the foundation for future exploration of type-associated differences in clinical presentations and biological properties.

## Introduction

Human rhinoviruses (HRVs) are highly prevalent respiratory pathogens, most commonly associated with mild upper respiratory tract disease and exacerbations of pre-existing respiratory disease such as asthma. They are also increasingly recognized as underlying more severe disease manifestations, such as bronchiolitis in young children and in the immunosuppressed. The increasing use of molecular methods for respiratory virus screening has contributed to this reappraisal of rhinoviruses, as has the recent discovery of an entirely novel rhinovirus group, refractory to previously used virus isolation methods but now known to be highly prevalent and widely circulating worldwide (Arden *et al.*, 2006; Kaiser *et al.*, 2006; Lamson *et al.*, 2006; Kistler *et al.*, 2007; Lau *et al.*, 2007; Lee *et al.*, 2007; McErlean *et al.*, 2007; Renwick *et al.*, 2007; Olenec *et al.*, 2010).

These newly characterized rhinoviruses have been proposed to belong to a novel species of rhinovirus (designated species C; HRV-C), recognizing their substantial sequence divergence from other classified species within the genus *Enterovirus* of picornaviruses (Carstens, 2010; Knowles, 2010) (Fig. 1a). Clinically and biologically, they share many attributes with the other designated HRV species, HRV-A and -B. Most studies of HRV-C disease associations, typically focused on children from asthmatic and/or hospital-based populations (Arden & Mackay, 2010), have demonstrated a similarly broad range of clinical outcomes to those observed in HRV-A and -B infections and, indeed, with other respiratory viruses. Some

A full list of all HRV-C variants characterized to date, categorized into a total of 61 confirmed or provisionally assigned types, is available with the online version of this paper.

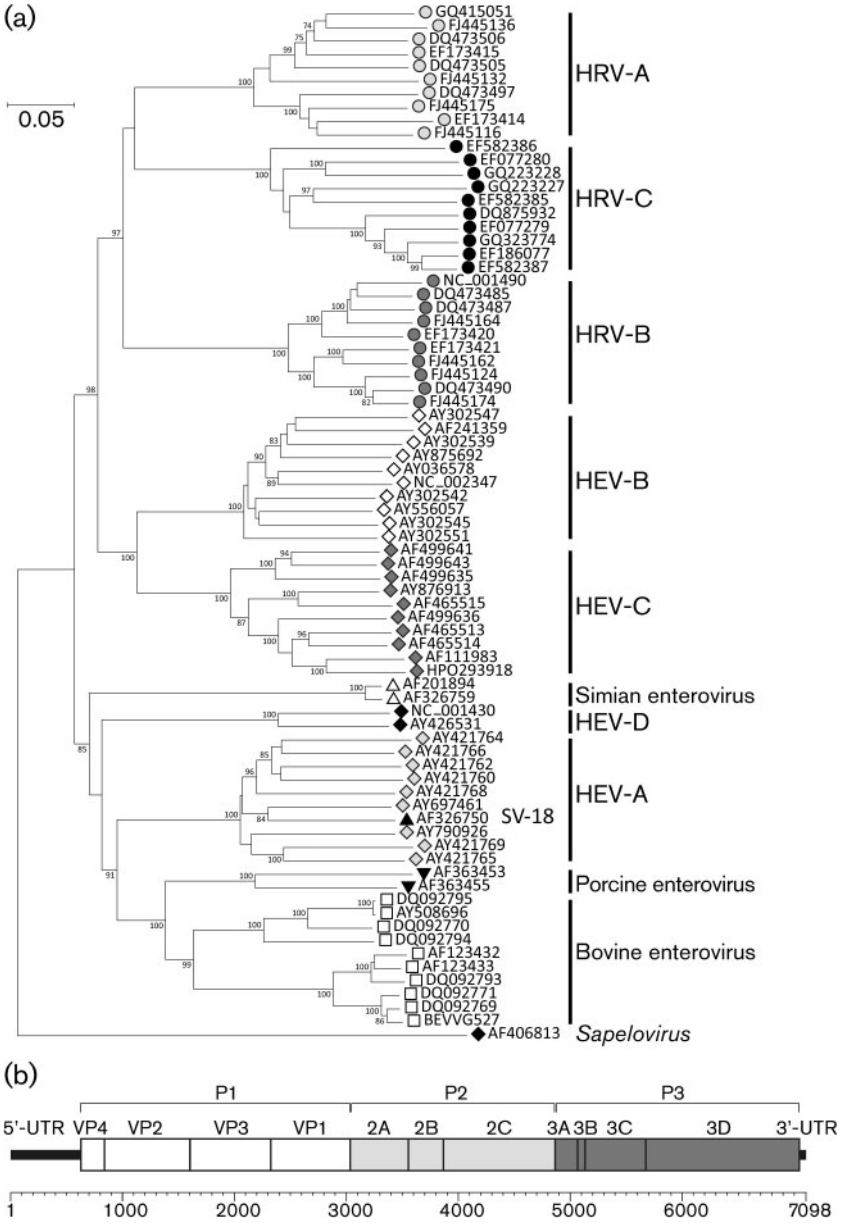


**Fig. 1.** (a) Sequence relationships between species currently assigned to the genus *Enterovirus*, depicted by phylogenetic analysis of aligned sequences from the P1 (capsid protein-encoding) region [positions 616–3125, numbered according to the reference sequence (GenBank accession no. EF582385); species identified by different symbols and shading]. The tree was constructed by neighbour-joining analysis of pairwise amino acid *p* distances, with branches showing $\geqslant$70 % bootstrap support labelled. The sequence of porcine enterovirus 8 (genus *Sapelovirus*) was used as an outgroup. For presentation purposes, only the 10 most divergent sequences were included for species containing more than 10 sequences (HEV-A, -B, -C, HRV-A, -B and bovine enteroviruses). Bar, amino acid *p* distance of 0.05. (b) Diagram of the rhinovirus genome, identifying the 5′- and 3′-UTRs and structural (P1) and non-structural (P2, P3) gene regions, along with the designations of their encoded proteins. The genome is drawn to scale, using the complete genome sequence 024 (GenBank accession no. EF582385) for numbering.

studies show no difference in clinical outcome between HRV species (Lau *et al.*, 2007; Piotrowska *et al.*, 2009), whereas others provide evidence for a more frequent role of HRV-C in lower respiratory tract disease, febrile wheeze in infants and toddlers, and asthma exacerbations in older children (Lau *et al.*, 2007; Khetsuriani *et al.*, 2008; Miller *et al.*, 2009a; Wisdom *et al.*, 2009b). Contrastingly, one study described a shorter duration of asthma symptoms and less cough than seen in HRV-A infection (Arden *et al.*, 2010a).

HRV-C shares a number of features of its genomic organization with other members of the genus *Enterovirus* (Fig. 1b). This includes an approximately 7100 base genome containing a single reading frame, the absence of a leader protein, a P1 region encoding four capsid proteins, a 2A gene encoding a *cis*-acting proteinase, followed by a series of non-structural proteins collinear with those of other enteroviruses, including 3D (which encodes the RNA-dependent RNA polymerase). HRV-C contains a type I internal ribosomal entry site that is structurally similar to and has short regions of striking sequence conservation with those of other enteroviruses. Members of the genus *Enterovirus* do, however, differ in other aspects of their genome organization. Most evident is the variability in the position of the *cis*-acting replication element. This is located at a homologous position within the 2C coding sequence in all four human enterovirus (HEV) species A–D, but is variable in position in each rhinovirus species [within 2A in HRV-A (Gerber *et al.*, 2001), VP1 in HRV-B and proposed to be located at the 5′ end of VP2 in species C (Cordey *et al.*, 2008)]. In marked contrast to the ease with which HRV-A and -B can be isolated, HRV-C has, to date based on the

published literature, been unculturable *in vitro* (Lau *et al.*, 2007; McErlean *et al.*, 2007). Historically, this hindered its discovery and has additionally precluded investigation of its antigenic variability, a feature characteristic of other HRVs and HEVs.

## Proposal aims

Genetic characterization of HRV-C amplified from clinical specimens has provided evidence for extensive heterogeneity in the VP4/VP2 region, the existence of two phylogenetically separate groups of sequences in the 5′-UTR (one resembling sequences found in species A rhinoviruses; Han *et al.*, 2009; Huang *et al.*, 2009; Savolainen-Kopra *et al.*, 2009b; Wisdom *et al.*, 2009a) and substantial sequence divergence throughout the genome of the 11 full-length HRV-C sequences obtained to date (Lamson *et al.*, 2006; Lau *et al.*, 2007; Kistler *et al.*, 2007; McErlean *et al.*, 2007; Huang *et al.*, 2009; Tapparel *et al.*, 2009b; Arden *et al.*, 2010b). Although we currently lack the means to classify HRV-C serologically (as has been achieved for other rhinovirus and enterovirus species), we recognize and are responding to the need to develop a classification system for this species. This will assist in organizing the rapidly accumulating sequence data currently being generated from virological and clinical studies, and allow assignment of uniform type descriptions that will enable comparison of genetic variants characterized in separate studies over time and across different geographical regions.

This process is potentially made easier by the evident similarities in the pattern of sequence divergence of HRV-C to other rhinoviruses and enteroviruses for which classification methods have been developed and standardized. For example, the large number of distinct genetic lineages identifiable by sequence comparisons in the VP4/VP2 region matches the diversity in this region shown by different serotypes of species A and B rhinoviruses (Lau *et al.*, 2009). These differences are mirrored in VP1 (Huang *et al.*, 2009; Wisdom *et al.*, 2009b; McIntyre *et al.*, 2010) and other exposed regions of the capsid that underlie the latter's putative antigenic diversity. In developing classification proposals for HRV-C, we have strived to develop criteria for type assignment that are consistent with the principles used for other rhinoviruses and enteroviruses, whilst acknowledging differences in its diversity, genetic history and biology.

## Classification of enteroviruses and rhinoviruses into serotypes

HRV-A and -B comprise a number of antigenically distinct viruses designated on the basis of their cross-neutralization properties *in vitro*, currently totalling 74 serotypes of HRV-A and 25 of HRV-B (Kapikian *et al.*, 1967). These totals incorporate minor adjustments to the original classification when HRV was subsequently characterized genetically (Kapikian *et al.*, 1971; Hamparian *et al.*, 1987). As examples, one of the classical HRV prototypes, HRV-87, was found to belong to the species HEV-D as a variant of HEV-68 (Blomqvist *et al.*, 2002). A strain referred to as Hanks was considered to represent a candidate new type

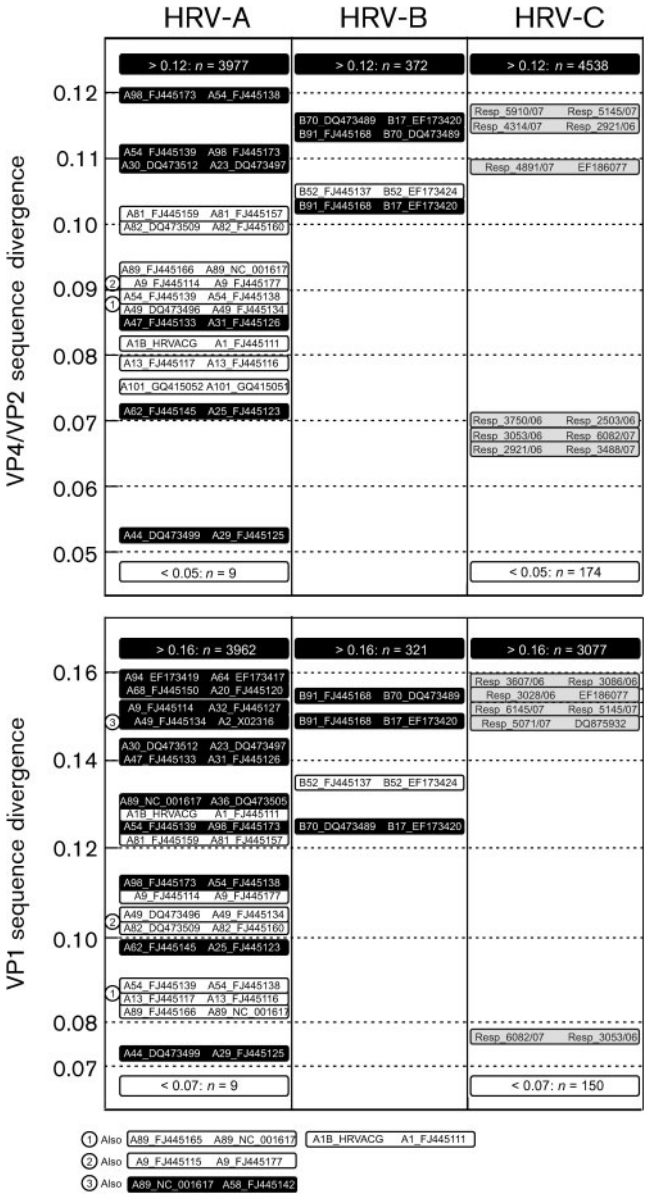


**Fig. 2.** Distributions of pairwise nucleotide *p* (uncorrected) distances (*y*-axis) between HRV-A and -B variants around the previously proposed thresholds (Savolainen *et al.*, 2002) dividing inter- and intra-serotype distances in VP1 (lower panel) and VP4/VP2 (upper panel). Pairwise comparisons in the equivalent distance range for HRV-C are shown for comparison. Unfilled boxes indicate pairwise distances between HRV variants previously classified as the same serotype; black-filled boxes, pairwise comparisons between different serotypes; grey boxes, pairwise comparisons of variants with unknown serological cross-reactivity (HRV-C). For clarity, multiple examples of pairwise distances between the same (sero)type pairs have not been shown.

but, on sequence analysis, it was found to be genetically similar to HRV-21 (Ledford *et al.*, 2004; Laine *et al.*, 2005).

In common with HEVs (Oberste *et al.*, 1999), there is a close correlation between sequence divergence of HRV-A and -B in the VP1 region (and other structural genes) and their designated serotypes (Savolainen *et al.*, 2002; Ledford *et al.*, 2004; Laine *et al.*, 2005). For HEVs, a nucleotide sequence divergence value of >25 % in VP1 (>15 % amino acid sequence difference) may be used as an alternative means to classify more recently discovered types without recourse to extensive serological characterization (Oberste *et al.*, 1999). Application of this principle has led to the assignment of 40 genotypically defined enterovirus 'types' in addition to the 64 traditionally classified serotypes. Thresholds of 12 % similarly differentiate inter- from intra-serotype divergences in the VP1 gene of species A and B rhinoviruses, respectively (Savolainen *et al.*, 2002; McIntyre *et al.*, 2010), providing the means in principle to detect novel species A types (e.g. Wisdom *et al.*, 2009b) without assaying for cross-neutralization (Savolainen *et al.*, 2002).

For rhinoviruses it is, however, recognized that some inconsistencies and overlap of divergence values between and within serotypes exist (Savolainen *et al.*, 2002). In species A, pairwise divergence values in VP1 between serotypes 95 and 8 (1.6 %), serotypes 44 and 29 (7.3 %), serotypes 62 and 25 (9.4 %) and serotypes 98 and 54 (11.4 %) are interspersed with those observed within serotypes, as is the pairwise distance between the species B serotypes 70 and 17 (12.3 %) (Fig. 2, lower panel). Overlaps in inter- and intra-serotype distances, often involving the same serotype pairs, are also evident from an equivalent analysis of pairwise distances of VP4/VP2 sequences (Fig. 2, upper panel). In two cases (95/8, 44/29), reanalysis demonstrated that these pairs did indeed show cross-neutralization (Cooney *et al.*, 1982; Ledford *et al.*, 2004), whereas one of the more divergent pairs, 62/25, did not (Cooney *et al.*, 1982). There is clearly scope to reinvestigate cross-reactivity of the other discrepant pairs. Overall, however, the actual distributions of pairwise distances between different serotypes of HEVs and rhinoviruses overlap minimally with intra-serotype nucleotide distances (as exemplified by HEV-B and HRV-A; Fig. 3a, b). For enteroviruses, the lowest value between these two distributions closely matches the type-assignment threshold now used for genotypic assignment of new

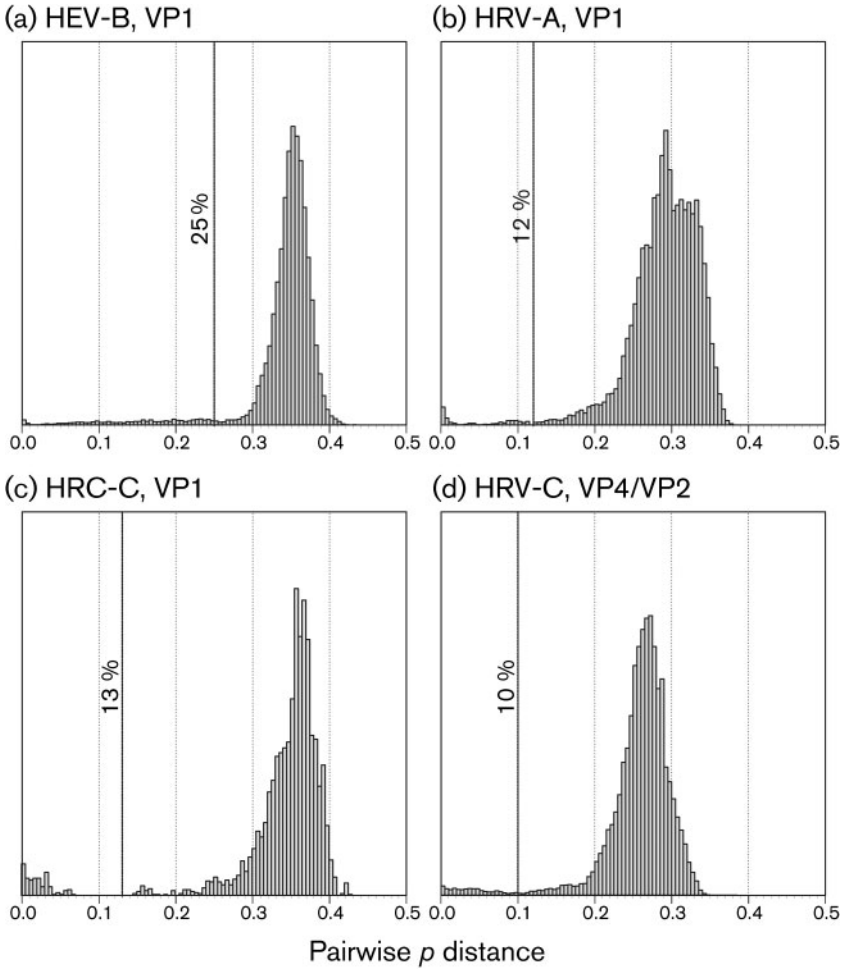


**Fig. 3.** Distribution of pairwise nucleotide *p* distances between: (a) available complete (>90 %) VP1 sequences of HEV-B ($n$=330); (b) available complete (>90 %) VP1 sequences of HRV-A ($n$=279); (c) available VP1 sequences of HRV-C ($n$=86; >90 % complete between positions 2304 and 3125); (d) all available VP4/VP2 sequences of HRV-C ($n$=702; >90 % complete between positions 615 and 1043). Previously designated (HEV) or proposed type thresholds are indicated by thick lines.

types. We propose to adopt this method for a genotypic classification of HRV-C.

## Rhinovirus recombination

The process of recombination creates chimaeric virus genomes in which different genome regions have separate evolutionary origins; recombinants may change in their phylogenetic relationships to other sequences between genome regions. For enteroviruses and rhinoviruses whose type assignments are dependent on the capsid genes (and the encoded differences in antigenic properties), regions that undergo extremely frequent recombination (such as the 5′-UTR, P2 and P3 non-structural gene regions in HEV) cannot therefore contribute to their (sero)type classification (Savolainen-Kopra *et al.*, 2009b).

Rhinovirus species A and B show much more consistent phylogeny relationships across the genome, as exemplified by the largely concordant phylogenies of the 3Dpol and VP4/VP2 regions (Savolainen *et al.*, 2004). There are, however, some inconsistencies evident on analysis of complete genome sequences of species A and B (Palmenberg *et al.*, 2009; Tapparel *et al.*, 2009a). For example, HRV-53 shows greater similarity to HRV-46 in the non-structural region than anticipated by their sequence relationship in the capsid-encoding region, whilst a similar comparison of HRV-78 and HRV-12 showed non-structural gene sequences to be more divergent. In these and other instances, most changes in phylogenetic relationship occurred at the P1/P2 boundary, implying separate evolutionary origins for the structural and non-structural gene blocks in some serotypes.

In contrast to species A and B, our recent extensive comparison of phylogenies of the VP4/VP2, VP1 and 3Dpol regions of species C demonstrated consistent branching orders and relative branch lengths in all three coding regions (McIntyre *et al.*, 2010). However, several phylogeny violations occurred between the 5′-UTR and VP4/VP2 trees, originating from a series of likely interspecies recombination events with breakpoints towards the 3′ end of the 5′-UTR (Han *et al.*, 2009; Huang *et al.*, 2009; Savolainen-Kopra *et al.*, 2009b; Wisdom *et al.*, 2009a).

Remarkably, most 5′-UTR sequences of species C cluster within the species A 5′-UTR clade, with the remainder being phylogenetically distinct (Han *et al.*, 2009; Huang *et al.*, 2009; Savolainen-Kopra *et al.*, 2009b; Wisdom *et al.*, 2009a). Those with species A-like 5′-UTR sequences have been named HRV-Ca, with the remainder assigned as HRV-Cc (Huang *et al.*, 2009). We have recently found that the region of 2A encoding the C-terminal domain of the proteinase also has a recombinant origin, with all available HRV-C sequences from this region clustering within the HRV-A clade (McIntyre *et al.*, 2010). The evolutionary events and the selection pressures underlying these instances of HRV-A/-C interspecies recombination are currently unknown.

## HRV-C heterogeneity and proposals for type assignments

Eleven (near-)complete genome sequences, 541 sequences from the VP4/VP2 region [>90 % complete between

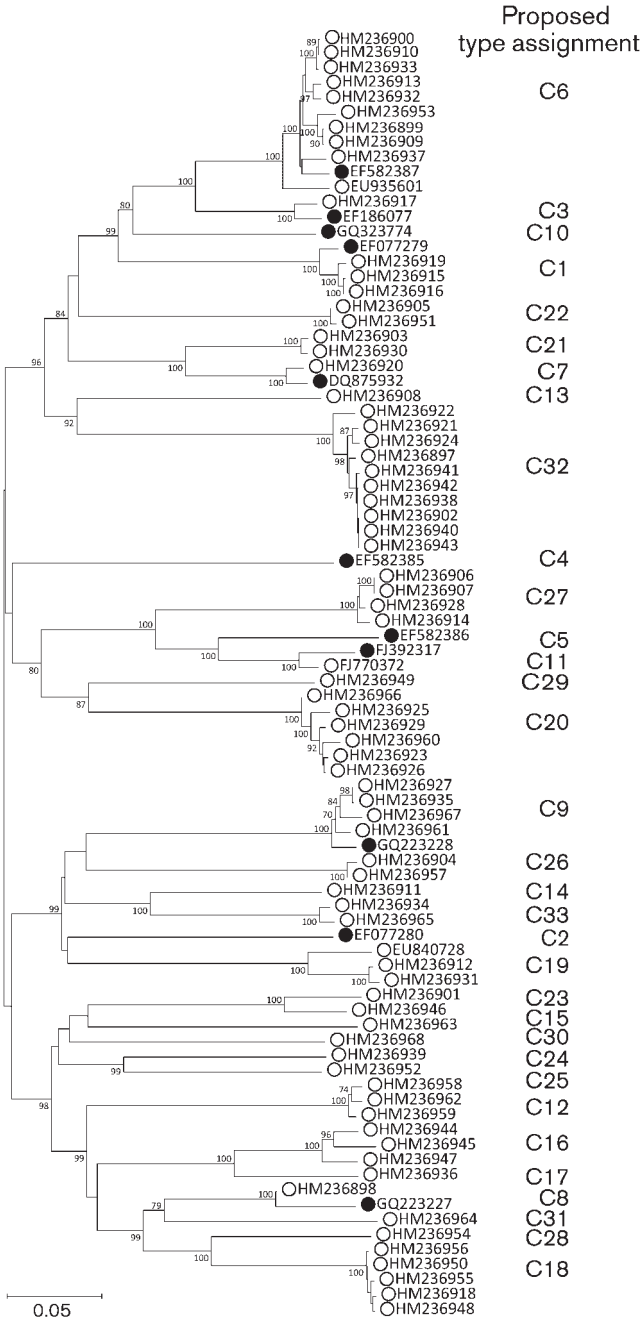


**Fig. 4.** Phylogenetic analysis of all available sequences of HRV-C in the VP1 region (>90 % complete between positions 2304 and 3125). The tree was constructed by neighbour-joining analysis of pairwise maximum composite likelihood distances implemented in the program MEGA (Tamura *et al.*, 2007); branches showing ⩾70 % bootstrap support are indicated. Complete genome sequences are labelled ●. Bar, maximum composite likelihood distance of 0.05.

positions 615 and 1043, numbered here and below using the complete genome HRV-C sequence 024 (GenBank accession no. EF582385)] from public databases and a further 160 unpublished sequences contributed by the authors of the current study, along with 86 complete VP1 (positions 2304–3125) and 89 partial 3Dpol (positions 6384–6854) sequences, collectively attest to the substantial genetic heterogeneity of HRV-C. The formation of a number of discrete clades of HRV-C in each genome region (Savolainen *et al.*, 2002; McIntyre *et al.*, 2010) (Fig. 4) supports the idea that genetic variants of HRV-C might be usefully classified into a number of types, comparable to types/serotypes of other HRV species.

The current lack of data on antigenic properties of HRV-C is unlikely to be addressed in the near future, due to difficulties with *in vitro* culture and the daunting task of creating and applying the necessary serotyping reagents should a viral culture system be developed. These factors necessitate a genotypic classification method. To investigate whether clear inter- and intra-type thresholds can be defined for HRV-C, we constructed a frequency histogram of the set of pairwise distances between all available sequences from the VP1 region (Fig. 3c) using previously described methods for constructing sequence alignments and determining sequence distances (McIntyre *et al.*, 2010). For comparison, we have additionally analysed an even larger dataset of available VP4/VP2 sequences (Fig. 3d).

As described previously (McIntyre *et al.*, 2010), the distribution of HRV-C VP1 sequence distances is indeed bimodal, with a clearly defined minimum (zero) value below 14.9 % and above 8 % (Fig. 2), which may be used as a threshold for putative assignment of HRV-C types. This corresponds closely to the 12 % threshold that divides within- and between-serotype distances in species A and B rhinoviruses (Savolainen *et al.*, 2002; McIntyre *et al.*, 2010). The distribution of pairwise distances in the VP4/VP2 region resembles that of VP1, with an equivalent minimum value corresponding to the type threshold of VP1 at 10 %. However, as a likely result of its shorter length and lesser degree of sequence diversity than VP1, the type threshold for VP4/VP2 was less clearly defined (Fig. 2). This pattern was also found in a similar comparison of VP4/VP2 region distance distributions in HRV-A and -B (McIntyre *et al.*, 2010) and in human enteroviruses (Oberste *et al.*, 1999; Mulders *et al.*, 2000).

## Type-assignment proposals

In formulating the following criteria for type assignment, we are aware of the need for simplicity and transparency in the assignment process and the use of criteria comparable to those used for genotypic classification in other enterovirus species. At the same time, these proposals should respect and adapt to differences in the pattern of diversity in species C and the occurrence of recombination. In addition, we acknowledge that current surveillance and genetic characterization of HRV-C are incomplete and we state the need for review of and, if necessary, revision of type-assignment criteria as further genetic data become available in the future. Finally, the use of genetic comparisons in restricted regions of the genome (VP1 and VP4/VP2) should not diminish perceptions of the importance of other genomic regions in shaping the phenotype of HRV-C. However, these, together with putative biological/epidemiological differences to be found in the future, lie specifically in the realm of research enquiry and we advise against their use as subsidiary or alternative classification criteria unless or until there is a future major reappraisal of our understanding of HRV diversity and genetics.

(a) A proposed HRV-C type should be phylogenetically distinct and show >13 % nucleotide sequence divergence in VP1 from all other

**Table 1.** Proposed assigment of complete genome sequences of HRV-C into types

| Type assignment | GenBank accession no. | Strain identifier | Submission | Variants | | Reference* |
|---|---|---|---|---|---|---|
| | | | | VP4/VP2 | VP1 | |
| HRV-C1 | EF077279 | NAT001 | 20 Oct 2006 | 17 | 4 | Kistler *et al.* (2007) |
| HRV-C2 | EF077280 | NAT045 | 20 Oct 2006 | 36 | 1 | Kistler *et al.* (2007) |
| HRV-C3 | EF186077 | QPM | 14 Dec 2006 | 12 | 2 | McErlean *et al.* (2007) |
| HRV-C4 | EF582385 | 024 | 27 Apr 2007 | 3 | 1 | Lau *et al.* (2007) |
| HRV-C5 | EF582386 | 025 | 27 Apr 2007 | 22 | 1 | Lau *et al.* (2007) |
| HRV-C6 | EF582387 | 026 | 27 Apr 2007 | 32 | 11 | Lau *et al.* (2007) |
| HRV-C7 | DQ875932 | NY-074 | 14 Jul 2008 | 4 | 2 | Lamson *et al.* (2006) |
| HRV-C8 | GQ223227 | N4 | 29 May 2009 | 6 | 2 | Huang *et al.* (2009) |
| HRV-C9 | GQ223228 | N10 | 29 May 2009 | 27 | 5 | Huang *et al.* (2009) |
| HRV-C10 | GQ323774 | QCE | 29 Jun 2009 | 7 | 1 | Arden *et al.* (2010b) |
| HRV-C11 | EU840952 | CL-170085 | 21 May 2010 | 11 | 2 | Tapparel *et al.* (2009b) |

*Reference for first submitted sequence for each type.

**Table 2.** Proposed type assigment of HRV-C variants represented by VP1 (and VP4/VP2) sequences

| Type assignment | VP4/VP2 region | | | VP1 region | | | References* |
|---|---|---|---|---|---|---|---|
| | GenBank accession no. | Submission date | Variants | GenBank accession no. | Submission date | Variants | |
| HRV-C12 | EF077264 | 20 Oct 2006 | 25 | HM236958 | 14 May 2010 | 3 | Kistler *et al.* (2007); McIntyre *et al.* (2010) |
| HRV-C13 | EU081795 | 3 Aug 2007 | 6 | HM236908 | 14 May 2010 | 1 | Renwick *et al.* (2007); McIntyre *et al.* (2010) |
| HRV-C14 | EU081796 | 3 Aug 2007 | 6 | HM236911 | 14 May 2010 | 1 | Renwick *et al.* (2007); McIntyre *et al.* (2010) |
| HRV-C15 | EU081800 | 3 Aug 2007 | 22 | HM236963 | 14 May 2010 | 1 | Renwick *et al.* (2007); McIntyre *et al.* (2010) |
| HRV-C16 | EU081808 | 3 Aug 2007 | 31 | HM236944 | 14 May 2010 | 3 | Renwick *et al.* (2007); McIntyre *et al.* (2010) |
| HRV-C17 | EU081809 | 3 Aug 2007 | 3 | HM236936 | 14 May 2010 | 1 | Renwick *et al.* (2007); McIntyre *et al.* (2010) |
| HRV-C18 | EU590074 | 25 Mar 2008 | 30 | HM236918 | 14 May 2010 | 5 | Savolainen-Kopra *et al.* (2009a); McIntyre *et al.* (2010) |
| HRV-C19 | EU697850 | 5 May 2008 | 9 | EU840728 | 20 Jun 2008 | 3 | Briese *et al.* (2008); Tapparel *et al.* (2009a) |
| HRV-C20 | EU697851 | 5 May 2008 | 14 | HM236923 | 14 May 2010 | 6 | Briese *et al.* (2008); McIntyre *et al.* (2010) |
| HRV-C21 | EU752377 | 26 May 2008 | 16 | HM236903 | 14 May 2010 | 2 | Miller *et al.* (2009a); McIntyre *et al.* (2010) |
| HRV-C22 | EU752381 | 26 May 2008 | 10 | HM236905 | 14 May 2010 | 2 | Miller *et al.* (2009a); McIntyre *et al.* (2010) |
| HRV-C23 | EU752424 | 26 May 2008 | 21 | HM236901 | 14 May 2010 | 2 | Miller *et al.* (2009a); McIntyre *et al.* (2010) |
| HRV-C24 | EU752426 | 26 May 2008 | 14 | HM236939 | 14 May 2010 | 1 | Miller *et al.* (2009a); McIntyre *et al.* (2010) |
| HRV-C25 | EU752427 | 26 May 2008 | 15 | HM236952 | 14 May 2010 | 1 | Miller *et al.* (2009a); McIntyre *et al.* (2010) |
| HRV-C26 | EU752441 | 26 May 2008 | 19 | HM236904 | 14 May 2010 | 2 | Miller *et al.* (2009a); McIntyre *et al.* (2010) |
| HRV-C27 | GQ223122 | 9 Jan 2009 | 14 | HM236906 | 14 May 2010 | 4 | Huang *et al.* (2009); McIntyre *et al.* (2010) |
| HRV-C28 | GQ223134 | 9 Jan 2009 | 11 | HM236954 | 14 May 2010 | 1 | Huang *et al.* (2009); McIntyre *et al.* (2010) |
| HRV-C29 | FJ615699 | 9 Jan 2009 | 4 | HM236949 | 14 May 2010 | 1 | Miller *et al.* (2009b); McIntyre *et al.* (2010) |
| HRV-C30 | GQ476669 | 13 Aug 2009 | 2 | HM236968 | 14 May 2010 | 1 | Wisdom *et al.* (2009b); McIntyre *et al.* (2010) |
| HRV-C31 | GU294380 | 4 Dec 2009 | 4 | HM236964 | 14 May 2010 | 1 | Wisdom *et al.* (2009b); McIntyre *et al.* (2010) |
| HRV-C32 | GU294466 | 4 Dec 2009 | 13 | HM236897 | 14 May 2010 | 10 | Wisdom *et al.* (2009b); McIntyre *et al.* (2010) |
| HRV-C33 | GU294480 | 4 Dec 2009 | 3 | HM236934 | 14 May 2010 | 2 | Wisdom *et al.* (2009b); McIntyre *et al.* (2010) |

*References for first submitted sequence for each type.

previously classified species C types. The VP1 sequence obtained for this sequence comparison must be >90 % complete between positions 2304 and 3125 for determining valid nucleotide sequence distances. The proposed threshold corresponds to approximately 8 % amino acid sequence divergence in VP1. However, for clarity and avoidance of conflicting assignments, we do not propose amino acid distances as an additional or alternative criterion for type assignments.

(b) A sequence from the VP4/VP2 region (between positions 615 and 1043) can be used for identification of HRV-C types among the much larger dataset of VP4/VP2 sequences that have been obtained from surveillance studies.

(c) Types should be numbered sequentially from 1 using a 'C' prefix to distinguish them from serotype designations of other HRV species. In the tables of assignments drawn up, numbering commences with the 11 (near)-complete genome sequences HRV-C1 to -C11 (Table 1), based on submission date to GenBank.

(d) Subsequent assignments have been made (HRV-C12 onwards) to genetic variants of HRV-C for which VP1 and VP4/VP2 sequences are available, again ordered by submission date of the first sequence in either VP4/VP2 or VP1 (Table 2).

(e) The remaining genetic variants of HRV-C for which only VP4/VP2 region sequences are available and which show >10 % divergence from other species C sequences in this region should be assigned as provisionally assigned types (designated 'pat'), e.g. HRV-C_pat1, HRV-C_pat2 etc. (Table 3). If and when VP1 sequence data are determined for at least one member of this provisionally assigned type, it can be added to the list of confirmed types and removed from the provisional list.

(f) A designated Expert Group takes responsibility for the future coordinated assignment of HRV-C types, including a reappraisal of the type assignment as more sequence data accumulate. This might perhaps be nominated by the ICTV Picornavirus Study Group and include Study

**Table 3.** Provisional type assignment of HRV-C variants represented by VP4/VP2 sequences only

| Provisional type | GenBank accession no. | Submission date | Variants | Reference* |
|---|---|---|---|---|
| HRV-C_pat1 | EF077256 | 20 Oct 2006 | 21 | Kistler *et al.* (2007) |
| HRV-C_pat2 | EF077260 | 20 Oct 2006 | 8 | Kistler *et al.* (2007) |
| HRV-C_pat3 | EU081790 | 3 Aug 2007 | 15 | Renwick *et al.* (2007) |
| HRV-C_pat4 | EU081791 | 3 Aug 2007 | 13 | Renwick *et al.* (2007) |
| HRV-C_pat5 | EU081799 | 3 Aug 2007 | 20 | Renwick *et al.* (2007) |
| HRV-C_pat6 | EU081802 | 3 Aug 2007 | 29 | Renwick *et al.* (2007) |
| HRV-C_pat7 | EU081803 | 3 Aug 2007 | 7 | Renwick *et al.* (2007) |
| HRV-C_pat8 | EU081805 | 3 Aug 2007 | 11 | Renwick *et al.* (2007) |
| HRV-C_pat9 | EU081807 | 3 Aug 2007 | 13 | Renwick *et al.* (2007) |
| HRV-C_pat10 | EU590054 | 25 Mar 2008 | 14 | Savolainen-Kopra *et al.* (2009a) |
| HRV-C_pat11 | EU590061 | 25 Mar 2008 | 5 | Savolainen-Kopra *et al.* (2009a) |
| HRV-C_pat12 | EU590064 | 25 Mar 2008 | 5 | Savolainen-Kopra *et al.* (2009a) |
| HRV-C_pat13 | EU697839 | 5 May 2008 | 6 | Briese *et al.* (2008) |
| HRV-C_pat14 | EU697852 | 5 May 2008 | 1 | Briese *et al.* (2008) |
| HRV-C_pat15 | EU743925 | 22 May 2008 | 10 | Dominguez *et al.* (2008) |
| HRV-C_pat16 | EU752358 | 26 May 2008 | 2 | Miller *et al.* (2009a) |
| HRV-C_pat17 | EU752398 | 26 May 2008 | 6 | Miller *et al.* (2009a) |
| HRV-C_pat18 | EU752412 | 26 May 2008 | 21 | Miller *et al.* (2009a) |
| HRV-C_pat19 | FJ598096 | 29 Dec 2008 | 5 | Currently unpublished |
| HRV-C_pat20 | FJ615722 | 9 Jan 2009 | 1 | Miller *et al.* (2009b) |
| HRV-C_pat21 | FJ615737 | 9 Jan 2009 | 4 | Miller *et al.* (2009b) |
| HRV-C_pat22 | FJ615745 | 9 Jan 2009 | 1 | Miller *et al.* (2009b) |
| HRV-C_pat23 | FJ841957 | 17 Mar 2009 | 2 | Calvo *et al.* (2009) |
| HRV-C_pat24 | FJ869923 | 27 Mar 2009 | 1 | Calvo *et al.* (2009) |
| HRV-C_pat25 | FJ869950 | 27 Mar 2009 | 4 | Calvo *et al.* (2009) |
| HRV-C_pat26 | GQ466482 | 7 Aug 2009 | 1 | Savolainen-Kopra *et al.* (2009c) |
| HRV-C_pat27 | GU214340 | 18 Nov 2009 | 5 | Piralla *et al.* (2009) |
| HRV-C_pat28 | HM347248 | 24 May 2010 | 1 | Currently unpublished |

*Reference for first submitted sequence for each type.

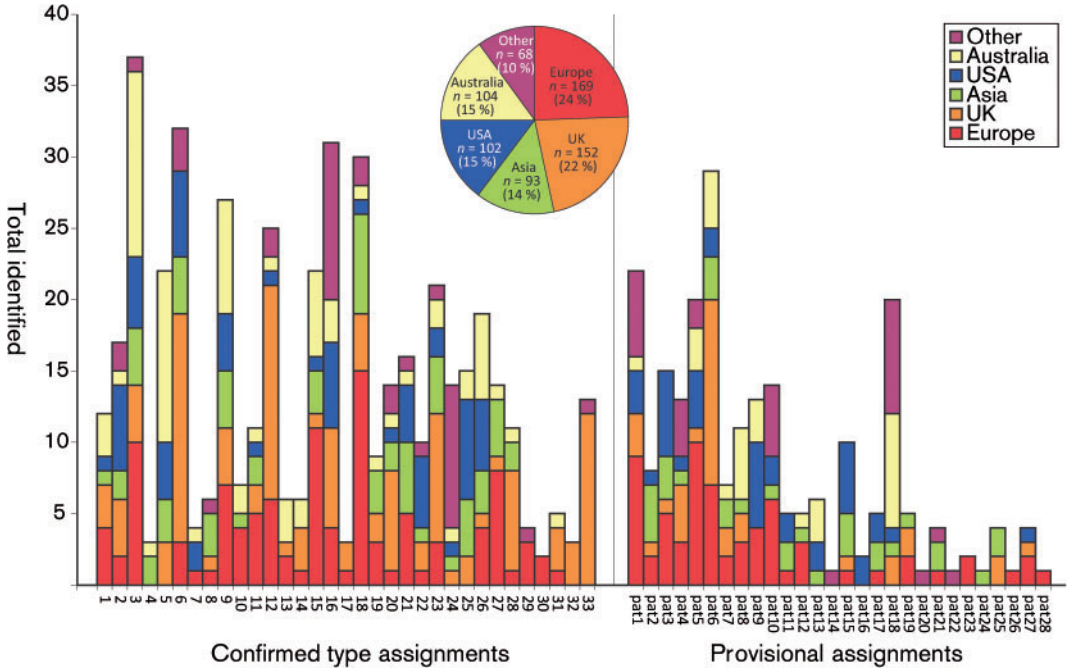


**Fig. 5.** Total numbers of each assigned HRV-C type identified by sequence comparisons in the VP4/VP2 region, divided by geographical region. The total representation of sequences from each geographical range is indicated by the inset pie chart.

Group members with expertise and experience in new enterovirus type assignments, as well as 'outside' scientists active in HRV-C or more general HRV research.

(g) Alignments of the VP1 and VP4/VP2 regions, along with information on the regions used for sequence comparisons, will be made available on a publicly available database accessible through the Picornavirus Study Group. These alignments will be regularly maintained and updated with new sequence data and type assignments as these become available.

(h) This Group should cooperate closely with those developing future type-assignment criteria for species A and B rhinoviruses to help ensure consistency in approach.

Applying these criteria to the currently available dataset of HRV-C sequences creates a total of 33 confirmed types (Tables 1 and 2) and a further 28 provisionally assigned types based on VP4/VP2 sequences (Table 3).

## Type identification

In drawing up specific classification proposals, we should emphasize that the process of type assignment is an activity distinct from type identification or detection. From the data obtained from genetic characterization of HRV-C in different genome regions and the lack of recombination observed (McIntyre *et al.*, 2010), we consider that identification of an HRV-C genetic variant as belonging to an already classified type can be achieved by sequence comparisons in VP4/VP2. Currently, most sequence data obtained for genetic characterization of HRV-C have been obtained in this region, including all of the confirmed types. These sequence data are derived from a wide geographical base, combining sequence data from Europe, USA, Australia, Japan and South-East Asia.

By phylogenetic analysis and examination of pairwise distances within the now-extensive dataset of VP4/VP2 sequences, the aforementioned threshold permits all HRV-C variants characterized to date to be categorized into a total of 61 confirmed or provisionally assigned types, the majority of which now contain multiple examples from geographically separate locations (Fig. 5; a full list of individual assignments is available as Supplementary Table S1 in JGV Online). The decreasing pace of identification of variants worldwide that can be assigned (even provisionally) as new types suggests a finite limit to the number that will eventually be classified. The actual total will, however, only become clear with more temporally and geographically widespread sampling.

In summary, this proposal draws together existing knowledge of the genetic diversity of HRV-C and applies principles established for type assignment of other enteroviruses to create a genotypically based classification scheme for HRV-C types. We hope that these proposals will be of value in future rhinovirus research, and provide the impetus to develop related type-assignment criteria for novel HRV-A and -B genetic variants that have been described.

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